Supplementary Material

for

A Regulator of *Dscam* Mutually Exclusive Splicing Fidelity

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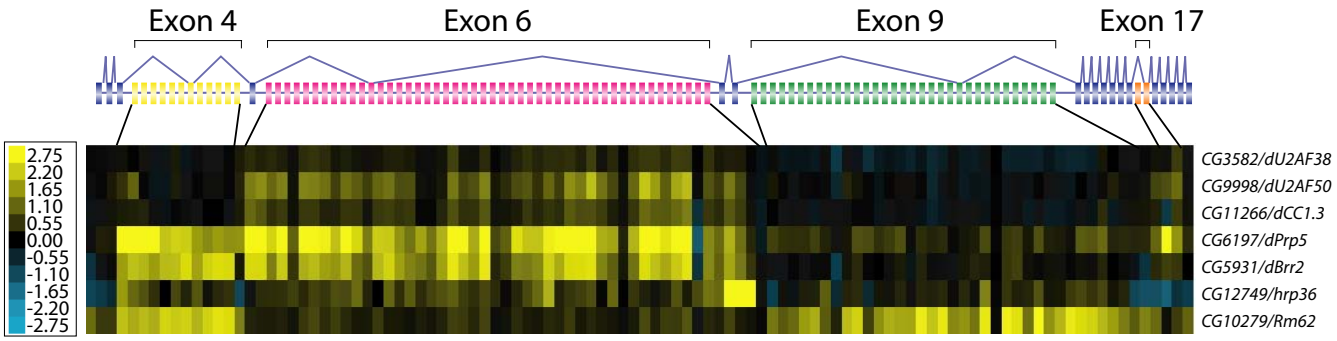
Supplementary Figure 1



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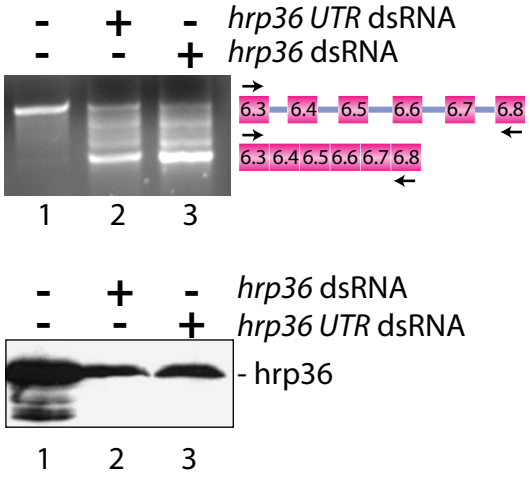
Supplementary Figure 1. Identification of proteins that have cluster wide impacts of *Dscam* pre-mRNA splicing. *Drosophila* S2 cells were treated with mRNA processing factor dsRNAs and total RNA from the cells was analyzed on custom high throughput microarrays. DsRNAs that affect multiple exons within one or more clusters, rather than just a specific exon within a cluster, are shown in the heat map. The probes for *Dscam* are shown in a 5' to 3' direction across the gene. The change in hybridization intensity for each probe is expressed as a *Z*-score where yellow indicates an increase in hybridization intensity and blue represents a decrease.

Supplemental Figure 2



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Supplementary Figure 2. Hrp36-dependent effects on exon 6 inclusion are not due to off-target RNAi effects. Above, two distinct *hrp36* dsRNAs, one corresponding to the coding region (lane 2) and the other to the 3'UTR (lane 3) have the same effect. Below, a western blot showing that the levels of hrp36 are equally reduced with both *hrp36* dsRNAs.

Supplementary Results

RNAi Screen

Supplementary Fig. 1 depicts a heat map of the dsRNAs that affect multiple exons within one or more of the *Dscam* alternatively spliced clusters. Some of these dsRNAs, such as *dU2AF* and *CG11266/CC1.3*, modulate specific clusters, as only exons within the exon 6 cluster are strongly affected. In contrast, others affect more than one clusters. For example, *CG5931/Brr2* and *CG6197/Prp5* strongly impact the splicing of both the exon 4 and 6 clusters, but not the exon 9 cluster, while *CG10279/Rm62/p68* affects the exon 4 and 9 clusters, but not the exon 6 cluster. Cells treated with *hrp36* dsRNA displayed defects in the splicing of all clusters to some extent, but splicing of the exon 6 cluster was the most strongly impacted (23% of the exon 6 variants had a *Z* score of > +/-1.0 in the *hrp36* dsRNA treated samples).

The proteins that modulate the splicing of the majority of exons within a cluster are the best candidates for regulators of the fidelity of mutually exclusive splicing. Due to the fact that the probes used in the microarray experiments correspond to sequences within the exons and did not span splice junctions, increases in the hybridization intensity of all probes within an entire cluster could be the result of several different events. For example, similar results could be obtained in the event of a general decrease in the splicing efficiency of all exons within a cluster, an increase in the stability of the excised introns (which contain multiple variable exons), or defects in the mutually exclusive splicing mechanism that would result in the inclusion of multiple variable exons in a

single mRNA. To differentiate between these possibilities, we performed a variety of RT-PCR experiments on RNA samples isolated from cells treated with these dsRNAs. These experiments revealed that depletion of dU2AF, dBrr2, dPrp5, dCC1.3, and Rm62 results in either an accumulation of unspliced pre-mRNA or an increase in the stability of the excised intron (data not shown). Although these results indicate that most of these proteins do not play a role in the mutually exclusive mechanisms, they do demonstrate that the splicing of each cluster has distinct protein requirements.